PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4318PTWO-ca	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/051711	International filing date *(day/month/year)* 04.08.2004	Priority date *(day/month/year)* 05.08.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant GENE DESIGN & DEVELOPMENT S.R.L. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.06.2005	18.08.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bradbrook, D Telephone No. +49 89 2399-7413

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/051711

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-17 as originally filed

Sequence listings part of the description, Pages

1-3 as originally filed

Claims, Numbers

1-15 as originally filed

Drawings, Figures

1-3 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/051711

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☒ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 11

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 11

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10,12-15
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10,12-15
Industrial applicability (IA)	Yes:	Claims	1-10,12-15
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.

PCT/EP2004/051711

Section III

1 Claims for which no international search report has been established have not been examined (cf Rule 66.1 PCT). Therefore, no opinion is provided with respect to the provisions of Art.33(1) PCT (i.e. novelty, inventive step and industrial applicability) for claim 11.

Section V

1 Reference is made to the following documents:

D1: PAPAFILI ET AL: "Common promoter variant in cyclooxygenase-2 represses gene expression: Evidence of role in acute-phase inflammatory response" ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY, vol. 22, no. 10, October 2002, pages 1631-1636

D2: CIPOLLONE ET AL: "Cyclooxygenase-2 polymorphism: putting a brake on the inflammatory response to vascular injury?" ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, vol. 22, no. 10, 1 October 2002, pages 1516-1518

D3: CIPOLLONE ET AL: "Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E2-dependent plaque instability" CIRCULATION, vol. 104, no. 8, 21 August 2001, pages 921-927

D4: CIPOLLONE ET AL: "Suppression of the functionally coupled cyclooxygenase-2/prostaglandin E synthase as a basis of simvastatin-dependent plaque stabilization in humans." CIRCULATION, vol. 107, no. 11, 25 March 2003, pages 1479-1485

D5: CIPOLLONE ET AL: "Cyclooxygenase-2 expression and inhibition in atherothrombosis." ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY, vol. 24, no. 2, February 2004, pages 246-255

D6: CIPOLLONE ET AL: "A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke" JAMA (JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION), vol. 291, no. 18, 12 May 2004, pages 2221-2228

2 The applicant's observations submitted with the letter dated 23.06.05 have been considered in establishing this report.

3 Novelty and Inventive step (Art.33(2) and 3) PCT)

3.1 The prior art does not disclose a method according to claims 1-10, a kit according to claims 12 and 13, or the uses as in claims 14 and 15 (cf also Section VIII). Therefore, said claims meet the requirements of Art.33(2) PCT.

3.2 However, the subject-matter of said claims is considered not to involve an inventive step as required by Art.33(3) PCT.

3.3 The SNP that is referred to in the claims, namely -765G>C in the COX-2 gene promoter, is known from the prior art. In vitro studies showed that the C allele causes lower COX-2 promoter activity, and patients with the C allele had lower plasma levels of C-reactive protein compared with patients carrying the G allele (D1: abstract). Although no direct association was proven, it was suggested in D1 that the -765C allele may be protective in cardiovascular disease in view of the known predictive function of raised CRP levels in cardiovascular events (D1: p.1635, col.1, para.2).

3.4 Expression of COX-2 has also been implicated in the clinical instability of atherosclerotic plaques in the context of simultaneous induction with PGE synthase by promoting plaque rupture induced by matrix metalloproteinases (cf D3: abstract), and inhibition of COX-2 expression has been related to a reduction of MMP activity and consequent plaque stabilization (cf D4: abstract).

3.5 Therefore, the suggestion that the -765G>C allele may be an important predictive tool for cardiovascular disease already exists in the prior art; furthermore, that it may be particularly relevant for conditions associated with plaque instability has also been indicated (cf also D2: p.1517-1518, bridging paragraph).

3.6 In the light of these disclosures, an inventive step could be acknowledged for the methods of the present application only insofar as they reflect the actual contribution in the art, namely that the presence of a C nucleotide at the SNP in question

indicates a lower risk to predisposition to certain types of cardovascular disease associated with the rupture of atherosclerotic plaques (cf present description, p.4, l.10-15 and p.13, l.30-32). This is particularly so in the light of the complex pattern of COX-2 activity, which renders it unreliable to generalise from a limited amount of data.

3.7 Accordingly, no inventive step can be acknowledged for claim 1, which does not specify the association between the particular allele and the risk, and further relates in general to cardiovascular diseases. One or both of the same deficiencies applies also to dependent claims 3, 4 and 9.

3.8 Dependent claims 2 and 5-9 do not appear to contain any additional features which, in combination with the features of the claims to which they refer, would render them inventive in the sense of Article 33(3) PCT. The feature of claim 2 is routine, and the features of claims 5-7 are known from D1, in which genotyping was carried out by AciI digestion of PCR products (D1: p.1632, col.2, para.2). Similarly, the primers used in claims 8 and 9, defined by SEQ ID Nos 3 and 4, correspond to the primers CF8 and CR8 disclosed in D1 (cf D1: Table 1).

3.9 For the same reasons as above, the use claims 13-15 are also not considered to involve an inventive step, contrary to the requirements of Art.33(3) PCT.

3.10 Although D1 does not disclose a kit, the packaging into a kit of two of the primers known from D1 for use in genotyping the polymorphism of interest is not considered to involve inventive activity. Furthermore, although D1 does not use the FauI restriction enzyme, this is considered to be a routine alternative that the skilled person would select without exercising any inventive skill. Therefore, the subject-matter of claims 12 and 13 does not meet the requirements of Art.33(3) PCT.

Section VI

1 The written opinion has been based on an assumed valid priority for the present application. Should the priority of the present application not be valid, D5 and D6 would be relevant with respect to novelty and inventive step (Article 33(2) and (3)

PCT).

Section VIII

1 Features following the term "optionally", used in claims 12 and 13, are considered to have no limiting effect on the scope of the claims (Art.6 PCT).

2 Claims 14 and 15 are unclear (Art.6 PCT): said claims refer to the use of the genotyping of the SNP at -765 "for the preparation of a prognostic test" respectively "to prepare diagnostic tests". These intended uses are vague, so that it is unclear if the genotyping is to be used as part of a method or as a basis for designing some sort of diagnostic kit. The claims have been interpreted as if they refer to the use of the genotyping method in a method of prognosis, respectively diagnosis.